IN THE UNITED STATES DISTRICT COURT
FOR THE NORTHERN DISTRICT OF OKLAHOMA

STATE OF OKLAHOMA,	)	
	)	
Plaintiff,	)	
	)	
v.	)	**Case No. 05-cv-329-GKF(PJC)**
	)	
TYSON FOODS, INC., et al.,	)	
	)	
Defendants.	)	

DECLARATION OF JIM C. LOFTIS, Ph.D., P.E.

I, Jim C. Loftis, Ph.D., P.E. hereby declare as follows:

1. Since January of 1979, I have been a faculty employee of Colorado State University and am currently serving as professor in the Department of Civil and Environmental Engineering. My educational background includes a Bachelor of Science degree in Agricultural Engineering from Oklahoma State University and Master of Science and Doctor of Philosophy degrees in Agricultural Engineering from Colorado State University in 1976 and 1978, respectively. I am a registered Professional Engineer in the State of Colorado.

2. I have taught at least 20 different courses at Colorado State University, focusing on water and the environment in courses such as Environmental Statistics and Nonpoint Pollution. To serve the professional community, I have taught short courses in Water Quality Monitoring Network Design and Environmental Statistics in the U.S., New Zealand, and Australia.

3. My faculty appointment as Colorado State University has involved a significant outreach and public education component through Cooperative Extension, and in 1990 my Extension activities in the area of agricultural impacts on water quality were recognized through an Outstanding Achievement Award from U.S. EPA Region VIII.

4. My research activities have focused in the area of environmental statistics, design of water quality monitoring networks, and agricultural nonpoint source pollution. I have also conducted significant research in the areas of water resource system optimization and irrigation management. My research sponsors have included the National Park Service, US Environmental Protection Agency, the US Department of Agriculture, the US Geological Survey, the US National Science Foundation, and IBM Corporation. My recent research and consulting activities have included the following: serving as one of three experts on an external review panel for "Statistical Analysis of Groundwater Monitoring Data at RCRA Facilities Unified Guidance", 2004 Draft, U.S. Environmental Protection Agency; the design of water quality monitoring networks for the Big Thompson Watershed and the Upper Cache La Poudre River for northern Colorado water providers; statistical evaluation of reservoir and stream quality monitoring data for Colorado Front Range water providers; development of modeling and monitoring approaches for managing selenium contamination in the Gunnison River Basin; and evaluation of salinity sources and trends in the lower South Platte River basin.

5. In September of 2007 I was retained by counsel for the State of Oklahoma to provide statistical expertise and assistance with their analysis of water quality monitoring data for the Illinois River Watershed. I have reviewed "Defendants' Motion to Exclude the Testimony of Dr. Valerie J. Harwood Pursuant to *Daubert v. Merrell Pharmaceuticals, Inc.*" I have reviewed the opinions of Dr. Valerie Harwood contained in

her report "Expert Report of Valerie J. Harwood, Ph.D." I have reviewed the opinions of Dr. Charles Cowan contained in his expert report "Rebuttal Report, Review of Principal Components Analysis of Data And Review of Inferences about Presence of Biomarkers in the Population of Animals from the Illinois River Watershed", November 26, 2008 and his deposition (Deposition of Charles Cowan, PhD, February 17-18, 2009). I have reviewed the opinions of Dr. Samuel Myoda in his expert report, "Report by Dr. Samuel Myoda". I have reviewed the draft paper "Identification of a Poultry Litter-Specific Biomarker and Development of a 16S rRNA Based Quantitative PCR Assay" by Jennifer L. Weidhaas, Tamzen W. Macbeth, Roger L. Olsen, Michael J. Sadowsky and Daniel Norat, and Valerie J. Harwood (2009). I received a copy of the biomarker vs. indicator bacteria data analyzed in Dr. Harwood's report and then in Dr. Myoda's report.

6. Defendants' motion, p. 15, asserts that "3. Dr. Harwood's Biomarker Process And Conclusions Are Inconsistent With Applicable Statistical Standards". Defendants base their argument largely upon the review of Dr. Harwood's expert report by their expert, Dr. Charles Cowan. Dr. Cowan claims that Dr. Harwood's sample sizes are too small to prove either the presence of the biomarker generally in poultry or its absence in other species. Both of these issues have been addressed since Dr. Cowan's review by the collection of additional data as reported in Weidhass, et al. (2009). However, two aspects of Dr. Cowan's review are particularly misleading and will continue to be so unless corrected.

7. In both cases, Dr. Cowan misrepresents what Dr. Harwood data says about the specificity of the biomarker method. Using Dr. Cowan's review as a basis, the Defendants' motion states (p.16) "Plaintiffs claim to have then proved the absence of the biomarker from other animals by testing for it in 13 cattle, two swine, five duck, five goose, and six human composite fecal samples. [Defendants' motion], Ex. 11 at 12-13. As Dr.

Cowan explains, these sample sizes are too small to support Plaintiffs' claim of having "validated" the "specificity" of the biomarker to poultry alone. "

8. The first problem with Dr. Cowan's review lies in the appropriate interpretation of specificity. Even though additional non-target samples have been collected, Dr. Cowan's interpretation of specificity would still suggest that there are too few. In Dr. Harwood's expert report, specificity is defined simply as the frequency of negative results when the contaminating source is absent. In statistical terminology, this could be referred to as the level of confidence or one minus the Type I error (false positive) rate. To determine the specificity of the biomarker using valid statistical sampling methodology requires a careful definition of the population of interest, as Dr. Cowan correctly points out. Unfortunately, however, the actual population of interest for application of the biomarker is the set of all possible uncontaminated samples (primarily water samples) to which the biomarker might be applied. Even if we restrict our definition geographically—to the IRW, for example—it is impossible to sample this population directly since there is no way to know which samples are contaminated and which are not. Therefore, the specificity of the biomarker in its intended application cannot (and should not) be determined directly by statistical sampling. What can and should be done instead is to apply the biomarker to very concentrated samples of feces, sewage, etc. from nontarget species that might be encountered in the actual population of interest and then evaluate the false positive rate for the non-target fecal samples. The actual false positive rate in the population of interest will be much lower than that for the non-target fecal samples since the biomarker method responds linearly to the log of concentration—see Figures 2 and 3 of Weidhass, et al. (2009)—and non-target feces will be diluted by a few orders of magnitude by the time contamination reaches streams.

9. In contrast with the above discussion, Dr. Cowan bases his opinion on an assumption that specificity must be demonstrated for each non-target species separately, with

adequate samples sizes for each species to show an acceptable false-positive rate on concentrated fecal samples. Clearly this is not possible since there are literally hundreds of non-target species. Nor is this necessary because this is not the definition of specificity that applies for practical application of the biomarker to samples of unknown composition. From basic probability theory, the probability of a false positive on a given sample is the product of the probability that the sample is contaminated by non-target feces times the probability of recording a false positive when the non-target feces are present. The latter probability is concentration dependent and will be much smaller when the biomarker method is applied to dilute samples in the field than when it is applied to concentrated samples of non-target fecal material in the laboratory.

10. It is, therefore, logical to define a "laboratory", as opposed to "field", specificity as one minus the false positive rate observed over a large collection of non-target fecal samples. This collection is constructed to represent the types of feces that would be encountered in the field. It is highly impractical and unnecessary to include all possible wildlife species in the collection, since the probability of a false positive from most of them is extremely small, as discussed earlier. This is the approach that Dr. Harwood has taken (although the term "laboratory specificity" is my own and is used simply for clarity in this declaration). The "laboratory" specificity that is reported in Weidhass, et al. (2009) based on 116 trials for non-target species is 108 (negatives) divided by 116, or 93 per cent. As discussed above, the large dilution of feces that occurs in the field insures that the actual field specificity will be much larger than the laboratory specificity.

11. The second problem with Dr. Cowan's review is in his interpretation of the single-species results for beef cattle. Although I have argued that the single-species laboratory results are not really essential for evaluating an overall field sensitivity, it is

probably valuable to look at the individual results for cattle. In Dr. Harwood's expert report, the beef cattle results were zero positives out of five trials. In his rebuttal report, Dr. Cowan states that based on zero positives out of 5 trials, the true incidence of the biomarker in beef cattle could be as high as 60%, and this is repeated in the Defendants' motion (p.16). Although Dr. Cowan offered no background or explanation, this statement is based on the binomial probability of observing zero positives out of five trials when the probability of a positive is 0.60. This probability, to three decimal places, is actually 0.0102. Therefore, the correct interpretation of the results is that if the true incidence of the biomarker in cattle feces were as high as 60% (and there is no measurement error), there is a 1.0000 minus 0.0102 or 99% probability that at least one positive would be observed in five trials. Since there were no positives observed, it is extremely unlikely that the true incidence in the beef cattle population represented in the trials is as high as 60%. Dr. Cowan's interpretation is, at best, extremely misleading.

12. Dr. Cowan's interpretation above also ignores the fact that each of the samples tested in Dr. Harwood's study was a composite of 10 sub-samples from a single field. Thus the effective sample size is certainly larger than five, even if some of the sub-samples came from the same animals. Although dilution of the biomarker is possible with compositing, the greatest possible dilution is by a factor of ten, which is much less than the dilution that will occur as the cattle feces are transported to a receiving water body on which the biomarker analysis would be performed in practice.

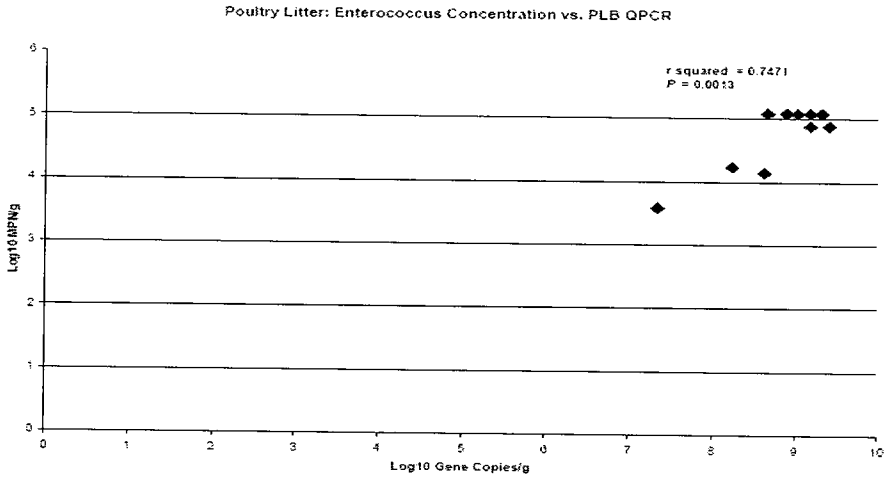
13. It is appropriate to correctly interpret the newer and more extensive results for beef cattle, reported in Weidhass, et al. (2009), which are zero positives out of 15 trials, again based on composite samples. If we take the most conservative approach of interpreting each composite as a single sample, the probability of observing zero positives in 15 trials when the true probability of a positive is 20% is 0.0352. In other words, if the fraction of the cattle

composite samples that had the biomarker were actually as high as 20%, there is a 96.5% probability that one or more of the samples would have tested positive (again assuming no measurement error). Thus, based on these 15 trials, we are quite (96.5%) "confident" that fewer than 20% of the composites contain the biomarker. Because the samples are composites, we are confident that many (as much as ten times) fewer than 20% of the individual animals excrete the biomarker. Furthermore, we are confident that many times fewer than 20% of false positives would occur in the field application of the biomarker assay to water samples that were greatly diluted compared to cattle manure. Thus the new results indicate that field specificity of the method against cattle manure is extremely high.

14. Defendants' motion (p.19) asserts that "5. Dr. Harwood's Biomarker Does Not Correlate with Indicator Bacteria". This assertion is based on analysis by their expert, Dr. Samuel Myoda, who claims that Dr. Harwood's sample size is too small and is flawed due to artificial truncation of data and a "suspect" data point. Dr. Myoda's statistical analysis and assertions about the lack of correlation between the biomarker and indicator bacteria, specifically enterococcus, in the population represented by Dr. Harwood's study are fundamentally incorrect.

15. There are three problems with Dr. Myoda's analysis. First and foremost, Dr. Myoda chose to re-analyze Dr. Harwood's data without using the log transform. If Dr. Myoda has experience with analysis of microbiological data, he knows that these data tend to be log normally distributed and that a log transform is essential in examining correlations. He should also know from Dr. Harwood's report, from which figure 4 is reproduced below, that Dr. Harwood's conclusions are based on log transformed data, as shown in the figure.

Figure 4. Correlation of enterococci concentrations with the poultry litter biomarker (QPCR) concentration.



(Reproduced from Harwood expert report, p.32). This failure to log transform these data caused Dr. Myoda to conclude that there was no correlation when in fact there was.

16. The other two problems follow from the first. Dr. Myoda claims that when right-censored values and the smallest observation, which he claims is suspect, are removed then the correlation disappears. However, much different results are obtained when the data are analyzed correctly, using the log transformation as Dr. Harwood did.

17. For example, consider the effect of the three right–censored or "greater-than" enterococcus observations at an MPN value of >120,000 per gram. "Greater-than" observations are extremely common in microbial data and occur when the actual microbial count in a sample is much larger than anticipated by the laboratory, and insufficient dilutions are performed at the outset. Thus the laboratory is able to determine only that the true value is greater than that which would result from the largest dilution performed. The resulting

"greater-than" data are not useless. They contain significant information, and the usual and conservative approach to dealing with them in statistical analysis is to substitute the lower limit, in this case 120,000, for which the log is 5.08. This is what Dr. Harwood did, and Dr. Myoda, who is doubtless familiar with "greater-than" data and their utility, is aware of that.

18. The censored and substituted data do not add correlation, and the observed correlation is not the result of censoring. In fact, censoring reduces the apparent correlation since the censored values are held constant at 120,000 (or 5.08 in log space), and their value, therefore, has no relationship or correlation with the biomarker level. In simple terms, the censoring puts a flat spot in the graph which would not exist if the uncensored microbial counts had been recorded. Thus the true correlation would be better than what is observed using the censored data. For Dr. Harwood's data, if one simply deletes the censored observations, the r-squared value for the remaining seven observations is 0.7517, a slight increase over the value of 0.7471 that Dr. Harwood found for the complete set of 10 observations. The p-value remains highly significant at p=0.001.

19. Dr. Myoda re-analyzed these same seven observations in Figure 4 of his expert report, reproduced below, without doing the log transform, thereby obscuring the correlation. With regard to his Figure 4, he claims that "… the correlation between "biomarker" and indicator bacteria is not strong enough to demonstrate any connection between poultry litter and indicator bacteria in the IRW." (Myoda expert report, p. 23, Exhibit 14 to Defendants' motion) However, had he analyzed these data in log space, as he must certainly know that he should, he would have obtained an r-squared value of 0.75 instead of the 0.29 that he shows in his Figure 4.

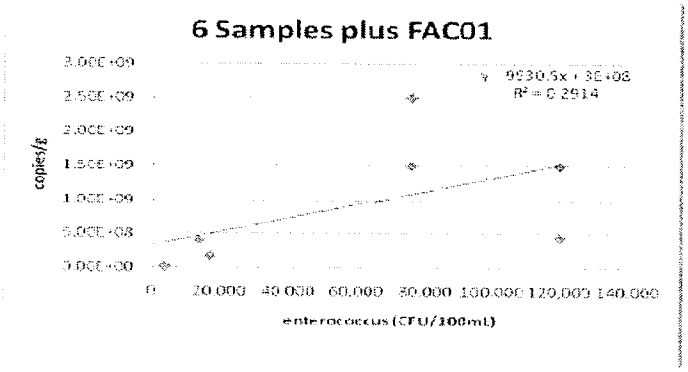


Figure 4. Six Original Litter Samples Plus FAC01

(Reproduced from Myoda expert report, p. 23, Exhibit 14 to Defendants' motion)

20. Dr. Myoda also analyzed these data with the smallest observation (sample #FAC01) removed, claiming that it is "suspect" and found an r-squared value of 0.1671 which indicates "no correlation". However, had he analyzed the data correctly, using the log transform, he would have found an r-squared value of 0.448. This result suggests that there could still be an important relationship, even though it is not statistically significant at the 90% confidence level (since p=0.146) given the very small sample size.

21. The Defendants' motion includes numerous quotes and references to the opinions of outside referees of Dr. Harwood's journal article manuscript. As an expert in the fields of environmental statistics and nonpoint source pollution, I have served many times on both ends of the review process (reviewer and author). I can say without reservation that many authors and technical reviewers who are truly experts in their own scientific disciplines are less well qualified to evaluate the statistical details of others (or even their own) work. Since we do not know who the cited reviewers are or anything about their qualifications in the area of statistics, their opinions on statistical matters should not be considered by the Court.

I declare under penalty of perjury, under the laws of the United States of America, that the foregoing is true and correct.

Executed on the 26th day of May, 2009.

Jim C. Loftis, Ph.D